From natural to artificial channels

Halža, Erik

Chapter 1
Introduction: From Natural to Artificial Channels

In the first chapter of this thesis, an overview is given of the efforts to synthesize and mimic natural membrane channels. Natural channel-forming structures and five groups of artificial channel-forming compounds are reviewed. Experimental techniques employed in the study of synthetic channel activity are discussed briefly as well as conditions for formation of channels in membranes. The answer to the question "What is the simplest molecule that can form a channel?" will be discussed. Finally, at the end of this chapter an outline of this thesis will be provided.

1.1 Introduction

The cell is the structural and functional unit of all known living organisms.[1] Each cell is able of self-containing and self-maintaining: it can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary.

All cells are surrounded by a closed membrane that defines the cell. Even within the cell, membranes play key roles in compartmentalization of biochemical products and processes.[2] These membranes prevent molecules generated inside the cell from leaking out and unwanted molecules from diffusing in; yet they also contain transport systems for carrying specific molecules inside and outside of the cell.

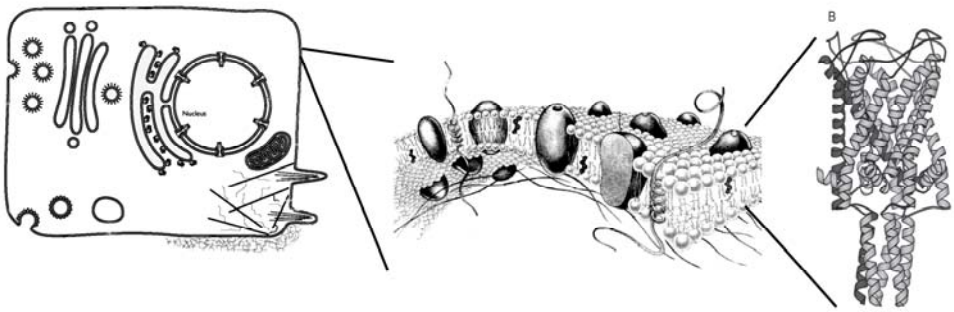


Fig. 1 *Structure of the cell with different compartments and the nucleus (left). Cell wall formed by lipid bilayer containing different proteins (middle). The structure of the MscL channel protein in the lipid membrane (right).*

Membranes are dynamic structures in which proteins swim in a lipid sea. The lipid components of the membrane form the permeability barrier and transport components act as a carrier of molecules in the form of pumps and channels.[3] Membranes are arranged as a bilayer "sandwich" where polar groups of lipids interact with the aqueous phase on both sides of the membrane and long alkyl chains form the "filling" of the sandwich. The thickness of the membrane is about 40 Å, too small to be seen under a microscope.[4]

The lipid bilayer of a membrane constitutes a hydrophobic barrier to a polar species such as cations and anions.[5] Transporting ions through this barrier, ion channels and pumps were created during the long time of evolution. Ion pumps and ion channels[1] fulfill different functions. The ion pumps transport ions against their

electrochemical gradient by using an energy source such as adenosine triphosphate (ATP) hydrolysis or the movement of another ion or substrate molecule. Ion channels, by contrast, are passive, simply catalyzing the movement of ions within their electrochemical gradient, in many cases at very high ion conduction rates (10^8 ions per second).[6] The flow of ions across the cell membrane is essential to many of life's processes. Ion pumps generate gradients across the membrane, which are used as an energy source by ion channels and other transport proteins to fulfill different life processes.

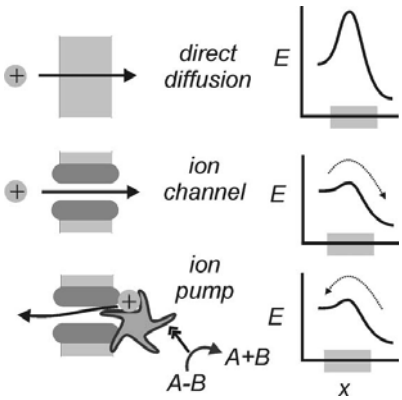


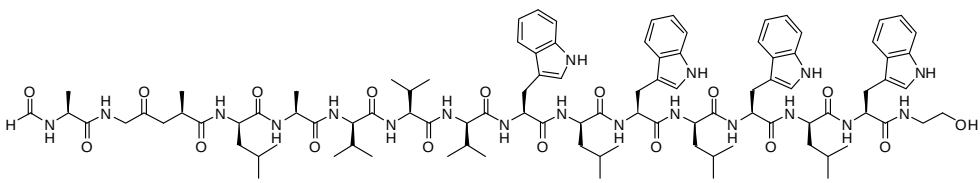
Fig. 2. *Cartoon of ion transport through a membrane by direct diffusion (top), or catalyzed by ion channel (middle). Both processes are driven by the concentration gradient of the transported ion. Ion pumps use reaction Gibbs energy changes (e.g. A-B bond breakage) to drive the transport of ions against a concentration gradient (bottom). Adopted from ref 2.*

Biological ion channels and pumps are typically large protein complexes consisting of a central channel portion that spans the membrane, and additional regions on one or both sides of the membrane that control access to the channel region.[2] Most transport proteins can transport certain ions across the membrane while at the same time excluding others. Ion selectivity can be extremely precise (for instance between Na^+ and K^+) and requires specific binding sites over at least part of protein length for the transported ions. These sites allow transport proteins to recognize ("feel") only the right ions. The recognition of the right ions requires dehydration (at least partially if not completely), and dehydration cost energy. Binding sites are compensating the energetic cost of dehydration by providing favorable compensatory interactions with the ion. Selectivity results when this energetic compensation is more favorable for one type of ion than for another, relative to the energy of dehydration.[6] For instance, in the bacterial KcsA channel (potassium channel)[7] a potassium ion

entering the channel from the top through a selective filter which is formed from carbonyl groups of the peptide backbone and a threonine OH group. The coordination sphere formed by these groups select potassium ions over sodium ions.

1.2 Ion Channels in Biological Systems

Supramolecular chemists have long been inspired by the functional sophistication of naturally occurring ion channels and ion pumps and a wide variety of biomimetic ion transport systems have been developed to replicate transport functions using small molecules and synthetic compounds.[8] The effort to design these structures came from two directions. One is to understand the function of natural channel systems by creating more simple systems and study their properties and the other is that natural transporters perform a number of cellular functions and processes that can be very useful in a technological and pharmaceutical context. In order to build artificial transport systems, it is important to know which structural framework an artificial transporter should have. The inspiration for this design can come not only from natural ion channels, but also from other classes of membrane-active compounds which can create pore-like structures through the membrane. Small molecules such as the antibiotics *valinomycin* and *lasalocid A* acts as ion carriers and as convergent donors for encapsulated ions.[8] The membrane-active small peptide such as *Gramicidin* represents another class of compounds that acts as components for ion channels. Gramicidin is a 15 α-amino acid peptide chain with alternating L- and D- amino acids. It forms a β-helical secondary structure with 6.3 residues per turn in one of the possible "active" ion channel forming structures (Fig. 3).[9] In the bilayer the peptide forms a head-to-head dimer of two right-handed single-stranded β-helices.[10] The resulting structure creates a water-filled tunnel through the membrane that is an efficient channel for alkali cations.



HCO-**L**-Val-Gly-**L**-Ala-**D**-Leu-**L**-Ala-**D**-Val-**L**-Val-**D**-Val-**L**-Trp-**D**-Leu-**L**-Trp-**D**-Leu-**L**-Trp-**D**-Leu-**L**-Trp-NH-CH_2-CH_2-OH

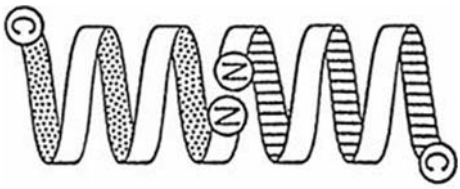


Fig. 3 *Structure of Gramicidin: sequence of α-amino acids (top, page 4), and head-to-head dimer of two helices (bottom).*

An interesting example is the group of *Peptaibols* compounds which are naturally occurring antimicrobial short peptides produced by fungi of the genus *Trichoderma*.[11] They form helices characterized by an N-terminal acyl group, a C-terminal 1,2-aminoalcohol, and a high content of the non-proteinogenic α-amino acid Aib (α-aminoisobutyric acid).[12] Long peptaibols such as *Alamethicin*, once in the plasma membrane, are proposed to pack together in parallel around a central ion-permeable pore, as inferred by the "helix-bundle" or "barrel-stave" models.[13,14] For more details about alamethicin molecule, see chapter 2 of this thesis.

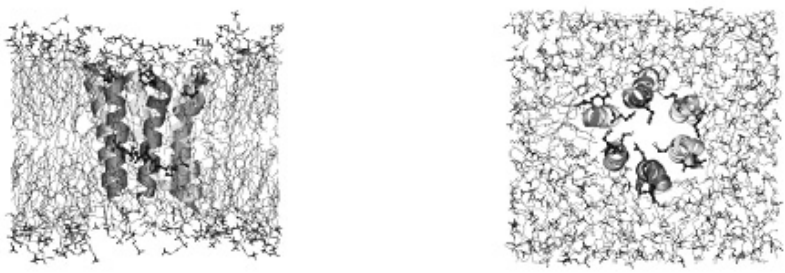


Fig. 4 *An alamethicin helix bundle consisting of 6 helices in a POPC bilayer. Side view (left), top view (right). Adopted from ref 14.*

The polyene antibiotic *Amphotericin* also forms channels in ergosterol-containing bilayer membranes.[15] The amphotericin monomer has dual amphiphilic character: the mycosamine head group orients the molecule in the bilayer membrane with its polar group in contact with the aqueous phase and the polyene tail, on the other hand, contact with the lipid hydrophobic region. Several monomers in this orientation aggregate to create a water-filled tube lined by the hydroxyl groups on the

edge of the amphotericin macrocycle. The presence of ergosterol is crucial for amphotericin activity. Ergosterol molecules act as "molecular matchers" that link the amphotericin monomers together (Fig. 5).[16]

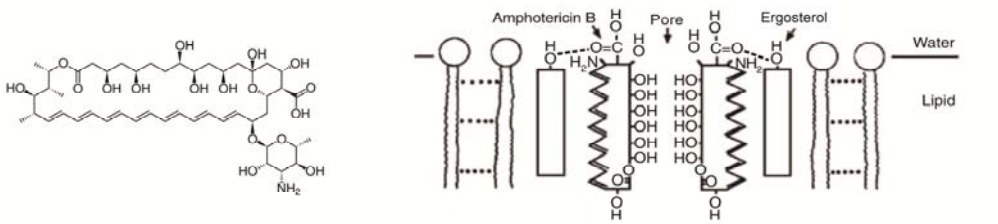


Fig. 5 *Structure of Amphotericin (left), and insertion of Amphotericin into a lipid bilayer together with ergosterol molecules (right).*

These examples of natural channel-forming structures show basic design criteria for artificial ion channels. First of all, the length of the structure or length of aggregates must match the thickness of the lipid bilayer (4 nm for the whole lipid bilayer or a length of 3-3.5 nm for the hydrophobic area of lipid bilayer). Secondly, the size of the artificial structure must enclose a significant volume for the passage of the ions. As a result the active structures have molecular weights of 3-4 kDa. Thirdly, the interior of the structure must be hydrophilic. This allows ion passage through the channel by ion-transporter interaction (as in the KcsA channel) or by transporter-solvated ion interaction (as in gramicidin, alamethicin). The fourth condition is that artificial systems have to incorporate themselves into the lipid bilayer by hydrophobic interactions with the hydrophobic lipid tails.[2]

1.2.1 Natural channels get artificial

Ion selectivity of biological ion channels is essential for fulfilling their function in cellular regulation and communication.[17] This requires the proper biological function of ion channels with respect to defined modes of control (gating). Channels are gated by a range of stimuli, including ligands, voltage, membrane tension, temperature, and even light. By controlling the gating process we can better understand the functions of the channels in living cells and if additional artificial

control mechanism is added, the channel can respond to more than one stimulus at the same time, for instance to a ligand and light.

Gramicidin A (gA) with β-helical[9] structure represents a suitable lead structure for ion-channel engineering.[18] Gramicidin A (gA) transports monovalent cations according to their dehydration energies (Eisenman I selectivity).[19] Permeability of the cations is decreasing with increasing dehydration energies. Koert and co-workers modified gA with crown ethers which is able to bind alkali-metal cations selectively, dependent on their size. Application of the planar lipid bilayer technique to this modified gA system showed that conductivities are of the same order of magnitude as for native gA. A higher preference for K^+ over Cs^+ was observed in comparison to the relative values for unmodified gA.[20] C-terminus modification of gA with a positive charge which can act as a stopper in the gA channel can dramatically influence ion selectivity as shown by Läuger[21], Woolley[22] and Koert.[23]

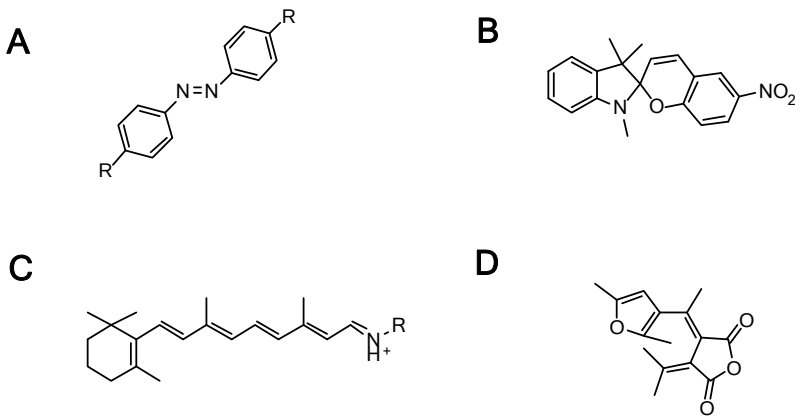


Fig. 6 *Photoswitches used in light-gated ion channels: azobenzenes (A), spiropyrans (B), retinal (C) and fulgides (D).*

Light-sensitive channels are other attractive candidates for ion-channel engineering because optical manipulation offers a high degree of spatial and temporal control. Over the last few decades, several channels have been successfully modified to be responsive to light, including the nicotinic acetylcholine receptor[24], gramicidin A[25], a voltage-gated potassium channel[26], an ionotropic glutamate receptor[27], and a mechanosensitive channel[29]. Although many channels have been modified, only a limited number of light-responsive switchable molecules have been introduced (Fig. 6, for detailed information about each switch molecule and its switching mechanism, see

chapter 2 of this thesis). Light-responsive molecules control the gating through a different mechanism, including changing the local electric field, moving blockers or agonists, or introducing conformational changes deep inside the protein.[28]

An illustrative example is modification of the *Mechanosensitive Channel of Large Conductance* (MscL from Escherichia coli) by attaching synthetic spiropyran switch molecule that undergoes light-induced zwitterion formation for reversibly opening and closing a 3-nanometer pore.[29] Normally the channel opens in response to tension, the introduction of polar or charged α-amino acids[30] or other charged compounds[31,32] into the 22nd amino acid position of MscL leads to spontaneous opening of the channel (for more information about MscL channel and side directed mutagenesis, see chapter 4 of this thesis). When the spiropyran molecule was introduced and after irradiation with long wavelength ultraviolet (366 nm) light the channel opened spontaneously in the absence of external pressure (Fig. 7).

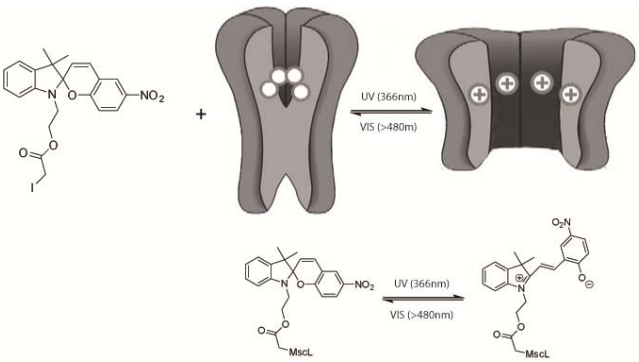


Fig. 7 *Schematic diagram of mechanosensitive channel of large conductance modified with a spiropyran molecule at the 22nd position of the amino acid chain (white cycle, top, the fifth subunit is removed) resulting in opening /closing of the channel. The photochemical reaction of spiropyran as a response to UV/Vis irradiation (bottom).*

1.3 How to study ion channels.

The functional analysis of ion channels requires the recording of single-channel current in the pico-ampere (pA) range or recording of the changes in the fluorescent intensity of a fluorescent dye. Three experimental techniques for the study of the ion channel activity are commonly used: fluorescent dequenching assay

(technique), planar lipid bilayer technique (black lipid membrane) and patch clamp technique. While the planar lipid bilayer technique together with the patch clamp technique give information about single-channel activity, the fluorescent dequenching assay gives information about assemblies of the channels incorporated in the liposomes.

The fluorescent dequenching assay is based on the formation of vesicles (or liposomes) prepared by dispersion of lipids in an aqueous buffer solution. Prepared spherical closed-shell vesicles typically have diameters of 20 nm to 1 μm.[33] The interior volume of the vesicles is of the order of femto- to attolitres. Release of this small amount of entrapped material into a large external volume can result in only relatively small changes, which requires sensitive detection methods.[2] Probably the most popular detection technique uses fluorescent dyes which are sensitive to changes in pH, to specific ions or are self-quenching at certain concentration. An important example is the *Calcein* dye which is often used to study vesicle stability and fusion of vesicles. Calcein[17] self-quenches at high concentrations (above 100 mM) but is fluorescent at low concentration. After encapsulation of calcein, at high concentration, in a liposome and removal of the non-encapsulated calcein, the leakage of calcein can be monitored by fluorescence spectroscopy (Fig. 8). Background fluorescence (a) at the beginning of the experiment is observed due to the fact that some calcein is still present outside of the liposome and also entrapped calcein has its background fluorescence. If the artificial channel is not present, fluorescence is constant during the whole period of the experiment (b). When a channel is introduced (c), an increase in fluorescent is observed due to leakage of the fluorescent dye out of the liposome, hence quenching occurs.

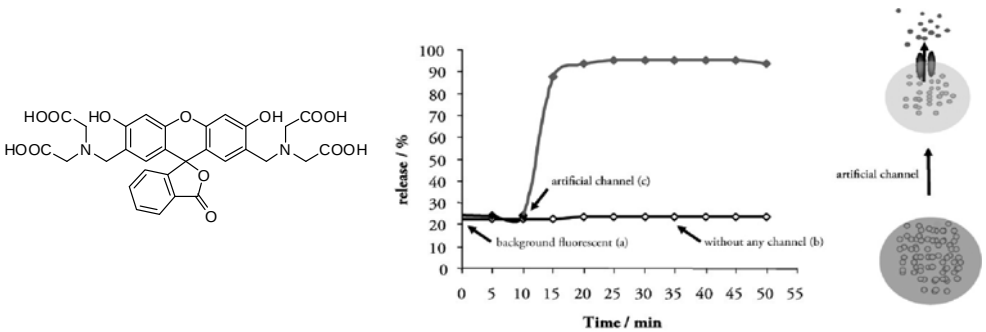


Fig. 8 *Structure of calcein (left) and a fluorescent dequenching experiment (right). Background fluorescence (a) is caused by residual fluorescence of entrapped calcein. Without any channel present (b) the background fluorescence is constant. If channel is added an increase in fluorescence is observed due to the release of calcein and subsequent dequenching effect.*

This technique is suitable for larger channels with large pore diameters because of the size of the dye is quite large (13 x 6 x 5 Å)[34] compared to the size of single alkali ions (3.8 Å for Na[35]).

The planar lipid bilayer technique is used in the study of the ionic conductance of a single ion channel, in which a constant transmembrane potential is applied and the resulting current changes are monitored as a function of time. A typical experimental setup for single–channel recording in a planar lipid bilayer is shown in Fig. 9.

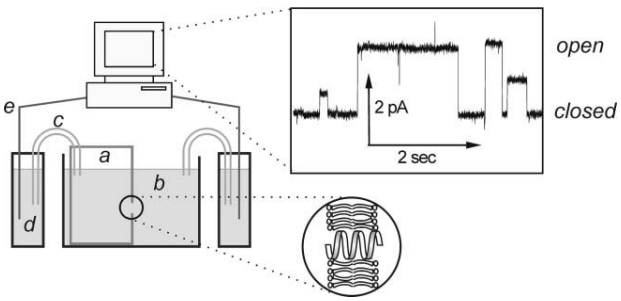


Fig. 9 *The black lipid technique. A cuvette (a), with a small hole cut into one face, is immersed in the electrolyte (b) and the bilayer is formed by painting across the hole. Electrical contact with the Ag/AgCl reference electrode (e) in the reference electrolyte (d) is via Agar salt bridges (c). Adopted from ref* 2.

A small hole between two compartments is covered with a lipid bilayer since the lipid membrane is a good insulator, at this stage low currents are observed upon the application of a voltage. When an ion channel is injected into one of the compartments and inserts into the lipid bilayer, a change in current is observed in the pA range in response to an applied voltage. This process appears as a step-change in the conductance of the lipid bilayer, representing a unique signature of the single ion channel. When the function of the channel is interrupted, the ionic current drops to the original value of the unmodified bilayer.

The patch clamp technique is routinely used technique in the studies of artificial channels, but numerous other applications are known including the study of the activity of channels in living cells. This technique was developed by Neher and Sakmann,[36] who were awarded the Nobel Prize for medicine in 1991 for this invention. By using this technique they demonstrated that ion channels are present in muscle fibers of frogs. For the patch clamp experiment a thin glass micropipette with a

diameter of thousands of a millimeter is employed and the current flowing through the pipette is measured. The pipette is pressed against the membrane and a seal is formed which can be monitored by optical microscopy.[37] Upon gentle suction inside the pipette the seal starts to enter the pipette and forms a so-called gigaohm seal (GΩ).[38]

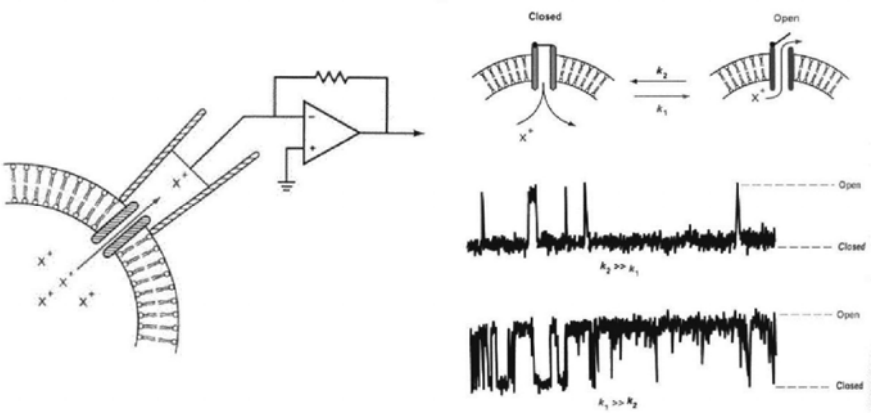


Fig. 10 *Schematic drawing of patch clamp experiment (left) and typical recorded diagram (right).*

This refers to the resistance of the seal, which is of the order of gigaohms (GΩ). A high resistance is necessary to reduce the background noise in the experiment. When the seal is in a fixed position in the pipette, the curvature can be varied by increasing or decreasing the suction. Release of the suction leads to flattering of the patch and an increase in suction induces curvature (Fig. 10).

1.4. Artificial channels

Inspired and challenged by biological ion channels and pores, synthetic chemists were trying to developed synthetic channels and pores to reproduce and mimic many of the fundamental functions of natural channels. In the past decades many different synthetic approaches were followed and used for the synthesis of artificial channels. All these attempts can be divided into several categories. Each category will be discussed shortly bellow.

1.4.1. Tubular synthetic ion channels in bilayer membranes.

Single molecular structures were used in the designs of early synthetic ion channels and pores[39] including crosslinking of barrel-stave supramolecules with central[40,41,42] or terminal hoops.[43,44,45,46,47,48,49] The crosslinking of macrocyclic crown ethers[44,45,50,51,52,53,54] and helical THF-peptides **1a** (tetrahydrofuran-peptides)[55] was successfully applied in the formation of active ion channels (Fig. 11).

The most studied and best characterized synthetic tubular ion channels are polyethers, e.g., crown ether. Crown ethers are well known in molecular recognition of cations.[56] Three different approaches have been developed in the design of cation channels based on polyethers: a cylindrical arrangement of crown ethers (Fig. 11, **1**), a polyether helix (Fig. 12, left, **2**) and a bundle of polyether strands (Fig. 13).[5]

One of the best characterized cylindrically-arranged crown ethers are a series of tris-macrocycles dubbed "*hydraphiles*" related to the active parent **1** (Fig. 11).[57] The original design involves co-facial stacking of all three crown ethers and creation of a transmembrane tube for cation conduction. However, the second macrocycle orients perpendicular to the plane of the other two and serves as ion relay, what is actually important in achieving the high activity of the channel.

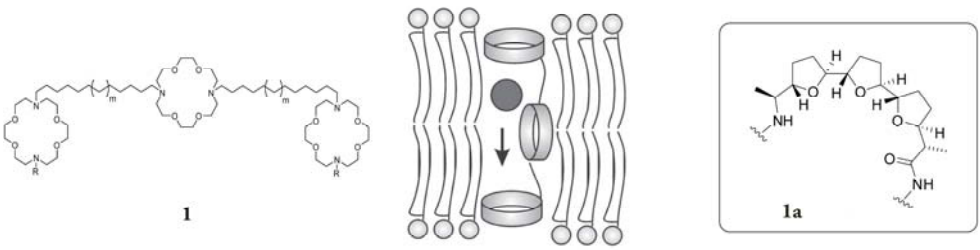
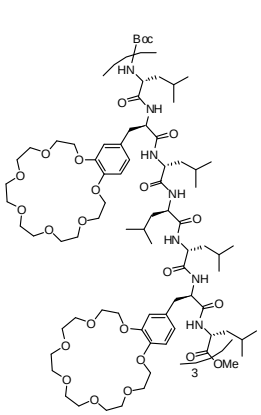


Fig. 11 *Structure of tris-macrocycle of crown-ethers (hydraphile **1**, left) and arrangement in lipid bilayer (middle). The structure of building block of the oligo-THF-peptide **1a** (right). Adopted from ref 2.*

The model for the structure **1** is supported by several studies including fluorescence quenching for the dansyl derivative (**1**, R=dansyl) and Hammett analysis of donor-acceptor properties of the benzyl derivative (**1**, R=benzyl) determined by ^{23}Na NMR linewidth experiment.[57] Antibiotic activity of "hydraphiles" with appropriate length against gram-positive and gram-negative bacteria was also found.[58]

The combination of oligo-leucine with an α-helical structure and the crown-ether modified phenylalanine in a [i, i + 4] fashion, positions crown ether moieties directly on top of each other forming desired tubular structure **2** (Fig. 12) which shows single channel activity in a planar lipid bilayer measurement.[59]



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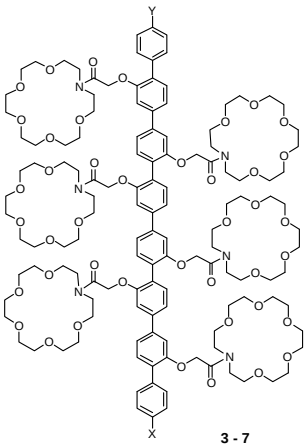


3 - 7

No.	Y	X	recognition
3	-SMe	-SMe	-
4	$-SO_2Me$	-SMe	+
5	-SMe	$-SCH_2CH_2NH_3^+$	-
6	$-SO_2Me$	$-SCH_2CH_2NH_3^+$	-
7	$-SO_2CH_2CH_2NH_3^+$	-SMe	+

Fig. 12 *Structure of crow-ether modified oligo-α-leucine-phenylalanine in [i, i+4] arrangement (**2**, left). An octiphenyl skeleton modified with crown ethers and various X and Y groups (**3-7**, right). The table shows activity of the octiphenyl structures depending on the X and Y substituents.*

Another example is the octiphenyl scaffold modified with crown ethers for an active tubular co-facial organized molecule.[39] The length of the molecule is controlled by the length of the scaffolds; on the other hand the face-to-face positioning of the crown-ethers cannot be controlled and depends only on the restricted rotation of the octiphenyl scaffold. Variable termini X and Y were placed on the ends of this system to form structures **3** – **7** (Fig. 12).

The vesicle assay (using a HPTS dye (8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt), Fig. 8) revealed that a molecule **4** with neutral X and Y groups is relatively ineffective as ion channel, but the molecules with charged groups (**5** and **6,** Fig. 12) showed channel activity. The push-pull rods **4** and **7** also mediate membrane depolarization more efficiently than ionophores **3**, **4** and **6**.[39,60]

Attaching ion-conducting side arms[5] to a cyclic core (crown ethers or cyclodextrines) is another way to demonstrate how crown ethers can be used in artificial channel design. An early example comes from the group of Lehn[61], who designed tetra-substituted crown **8** (Fig. 13) and a second example is from the Fyles[62] group featuring hexa-substituted crown ether **9** (Fig. 13). These types of compounds

belong to a large group of supramolecular aggregate based ion channels which will be discussed in more detail further on.

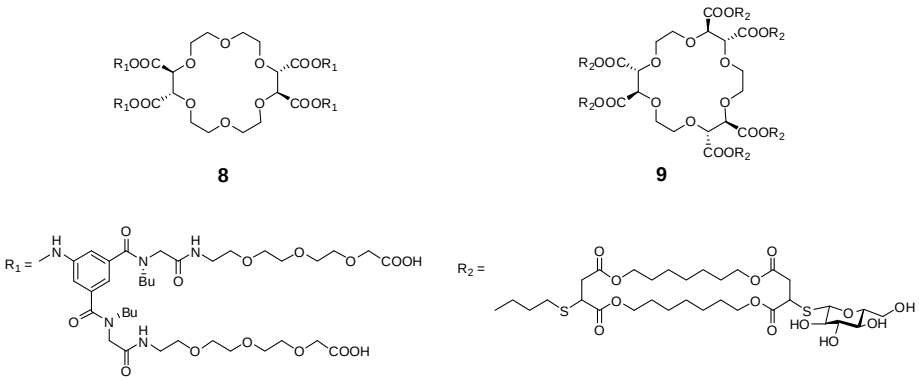


Fig. 13 *Modified crown ethers with various side chains (**8**, **9**) belong to the group of supramolecular aggregate based ion channels.*

1.4.1.1. Tubular ion channels based on octiphenyl β-barrel

The octiphenyl scaffolds are central to the formation of another class of tubular channels called rigid-rod β-barrels.[39] The cylindrical self-assembly of β-barrel pore **11** is due to preorganization by the non-planarity of p-octiphenyl staves in the octapeptide-p-octiphenyl monomer **10** (Fig. 14).[63]

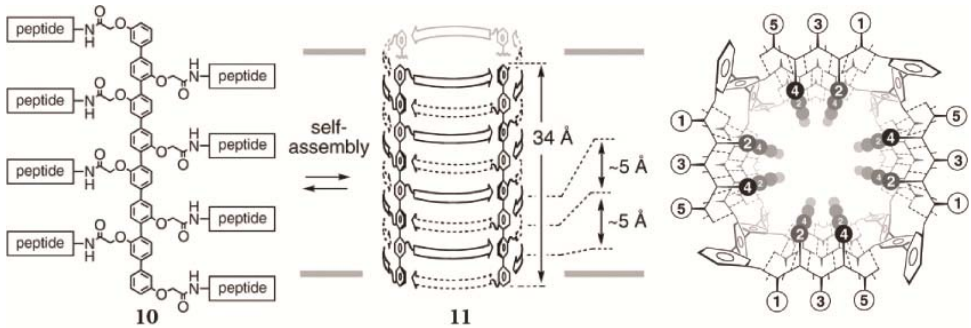


Fig. 14 *Design of peptide modified octiphenyl (**10**, left). Space arrangement with the diameters. The size of the structure is 34 Å, diameter between two peptide chains is 5 Å (**11**, middle). Top view with α-amino acid sequence. Positions 1, 3, 5 are hydrophobic α-amino acids and positions 2 and 4 are hydrophilic α-amino acid (right) Adopted from ref* 63.

The octiphenyl frame contains short chains of peptides forming a β-sheet oriented in anti-parallel fashion in a [i, i + 3] position on the octiphenyl.

The β-sheet orients α-amino acids in opposite sides of the sheet (Fig. 14). The active channel is formed by four octapeptide-p-octiphenyl monomers **10** stabilized by hydrogen bonding between anti-parallel interdigitated α-amino acid chains (Fig. 14, middle, **11**).

Rational and variable funtionalization of the outer as well as the inner pore surface (variations in α-amino acid sequence) can lead to different properties of the pore. Modification of the length of the peptide chains influences the activity of the barrel as well as its physical properties. Five α-amino acid residues form longer-lived barrels/pores (openings in the range of minutes) and with internal diameters of about 2.5 nm compared with three α-amino acid residues where the openings are in the range of seconds to milliseconds. In principle the internal pore design determines the nature of the interactions between the synthetic multifunctional pore and the molecules that pass through the pore across the bilayer membrane.

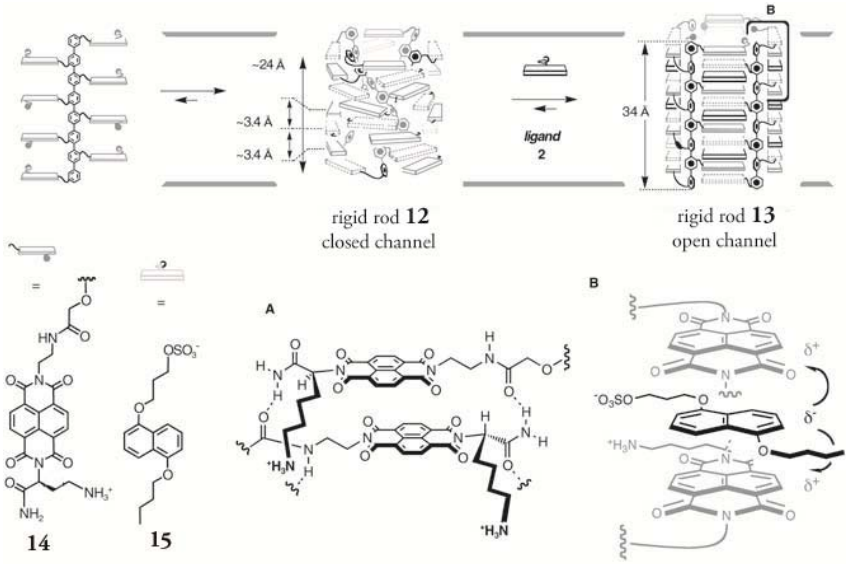


Fig. 15 ***Top***: *An octiphenyl core modified with π-stacking naphthalenediimides* ***14*** *induces twist in the structure. The gap between the two naphthalenediimides is 3.4 Å (****12****, top). When a donor molecule* ***15*** *is introduced an open channel forms (****13****) and an ion current is observed.* ***Bottom****: Schematic illustration of two naphthalenediimide moieties on top of each other (****A****) and intercalation of a dialkoxynaphthalene guest molecule (****15****) resulting in formation of an active channel (****B****). Adopted from ref 65.*

Many different guest molecules were examined and include carbohydrates, inositols, nucleotides, fullerenes, calixarenes and pyrenes, macromolecules such as polypeptides, polysaccharides, oligonucleotides and B-DNA. This design was used as an artificial tongue to sense different molecules in food.[64]

A π-stack architecture, so fundamental and important for DNA but absent in biological and synthetic ion channels, was introduced by simple replacement of the β-sheet in the rigid-rod β-barrels by π-stacking molecules, such as naphthalenediimide **14**. This leads to a twist in the structure of the p-octiphenyl stave and formation of an π-helix (Fig. 15). The distance between two sheets changes from 5 Å to 3.4 Å and the length changes from 34 Å to 24 Å (see Fig. 14, structure 10 and Fig. 15, structure 12).[65] The formation of a π-helix like structure has as a consequence that the channel becomes inactive. If a small guest (dialkoxynaphthalene, **15**) is introduced a charge transfer complex is formed, the twisted structure is broken and channel function is reestablished (Fig. 15). To ensure that π-stacks will be formed instead of linear self-assembled polymers, four principles were considered. Positioning of adaptable chiral biphenyls with a torsion angle $\omega \neq 0$ per stave, prevents p-octiphenyl rods from self-assembling into supramolecular polymers. Amides were placed at both ends of the naphthalenediimide stacks oriented parallel to the staves. The resulting hydrogen-bonded chains will position the π-stack in the barrel-stave arrangement. Internal crowding was expected to promote the self-assembly of higher hollow oligomers by steric hindrance between bulky groups of naphthalenediimides at the inner surface.

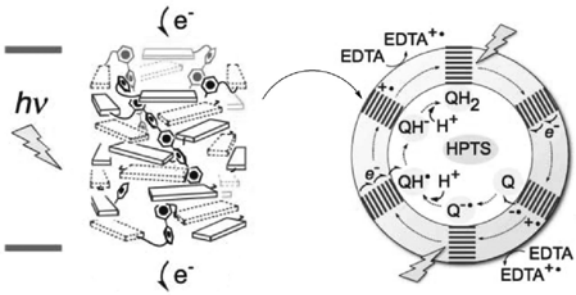


Fig. 16 *A photo-active system based on the octiphenyl naphthalenediimide structure (left) and the chemical pathways in the liposome experiments (right). Electron gradients formed after irradiation of the p-octiphenyl rods reduce quinone (Q) into QH_2 while consuming protons resulting in an increase in pH which was detected by pH sensitive fluorescent dye HPTS. Adopted from ref 66.*

This was achieved by attaching alkylammonium tails which were partially protonated. Internal charge repulsion promotes the self-assembly as well. Considering all these features, a π-stack systems like **13** (Fig. 15) can be formed.

By incorporation of blue and red-fluorescent naphthalene diimides into the p-octiphenyl rods in lipid vesicles containing quinone (Q) as electron acceptors and surrounded by ethylenediaminetetraacetic acid (EDTA) as electron donor, leads to an pH increase through quinone reduction upon excitation with light (Fig. 16).

After 635 nm irradiation of the externally added p-octiphenyl rods, electron flow through the phosphatidylcholin (PC) membrane was generated resulting in quinone reduction and an increase in the pH inside the vesicle which was detected by using a pH-sensitive fluorescent dye HPTS (8-hydroxypyrene-1, 3, 6-trisulfonate). [66]

1.4.1.2. Tubular ion channels based on D, L-α-amino acids

Pioneering work on artificial tubular channel systems maintained by peptide hydrogen bonding was performed by Ghadiri and co-workers.[67]

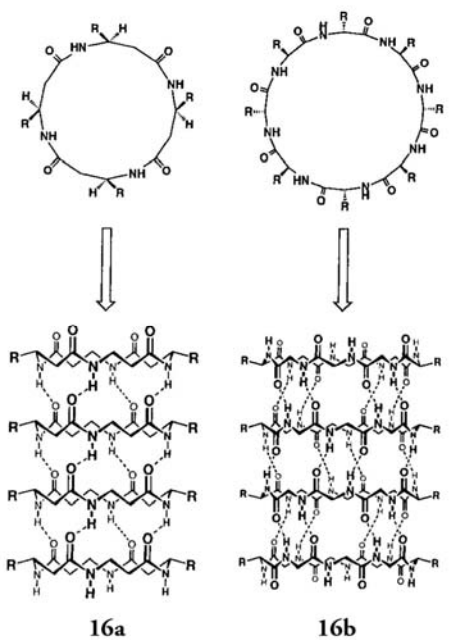
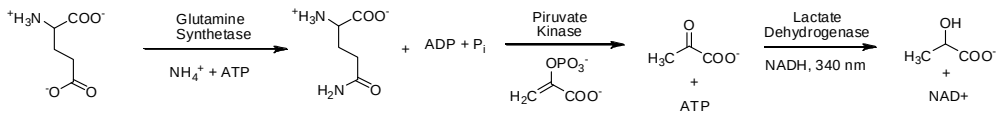


Fig. 17 *Structures and 3D representation of cyclic α-octapeptide (16a, left) and cyclic β-decapeptide (16b, right). Adopted from ref 67.*

The system typically contains cyclic α- or β- octa- or decapeptide **16a** and **16b** with an alternating D- and L-α-amino acid sequence.[68,69] Cyclic peptides were designed to adopt flat ring shaped conformations and stacks through extensive backbone-backbone hydrogen bonding to form tubular structures (Fig. 17).

Compound **16a**, where the substituent R is isopropyl and tryptophan, exhibited remarkable ion transport activities with single-channel K^+ conductance of 56 pS which was determined in liposome-based proton transport assays and by single channel conductance experiment.[68] It was demonstrated that octapeptides can transport ions selectively, and in the case of decapeptides they can transport small molecules such as glucose or glutamic acid. In the case of glutamic acid transportation, decapeptides were incorporated into the vesicles. Channel-mediated glutamic acid transport from large unilamelar vesicles to the external solvent under isotonic solution conditions was monitored by an enzymatic assay operating in the extracellular medium (Scheme 1).



Scheme 1 *Transport of glutamic acid through cyclic decapeptide in liposome monitored by enzymatic degradation of glutamic acid and subsequent NADH oxidation measured at 340 nm.*

This spectrophotometric assay couples glutamine synthetase activity with the reactions catalyzed by pyruvate kinase and lactate dehydrogenase by monitoring NADH oxidation at 340 nm.[70]

Compound **16b** with different R groups was also examined for antibacterial activity against gram-positive and gram-negative bacteria.[71] The antimicrobial action is rapid, suggesting that the toxicity is related to membrane depolarization rather than by a receptor-mediated site of action.

It is known that this cyclic peptide structure is not electrically conductive.[72] By introducing four cationic 1,4,5,8-naphthalenetetracarboxylic diimides (NDI) to the side chains of the octapeptide (**16a**), redox-triggered self assembly in aqueous solution can be achieved.

The peptide nanotubes (channels) formed by redox reaction of NDI are possessing highly delocalized electronic states (Fig. 18).[73] The mechanism of the self-assembly of monomeric units includes the reduction of the NDI moieties by mild chemical or electrochemical methods resulting in radical anions that are stable in the absence of oxygen.

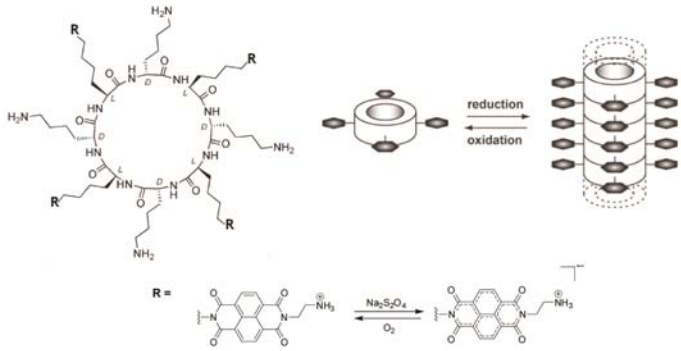


Fig. 18 *Cyclic octapeptide modified with four 1,4,5,8-naphthalenetetracarboxylic diimides undergoes redox-triggered self assembly in aqueous solution to form conducting peptide nanotubes. Adopted from ref 73.*

Furthermore, it is known that the NDI anion radicals self assemble in aqueous solution.[74] This leads to the formation of nanotubes which are a few hundreds of nanometers long and have diameters of 2 to 3 nm.[73]

1.4.2. Calix[n]arenes and cucurbit-based artificial channels

Resorcin[n]arenes (**17** and **18,** Fig.19), calix[n]arenes (**19**, Fig.19), and

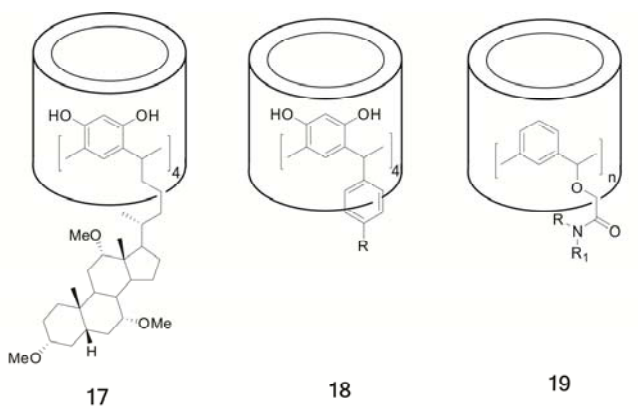
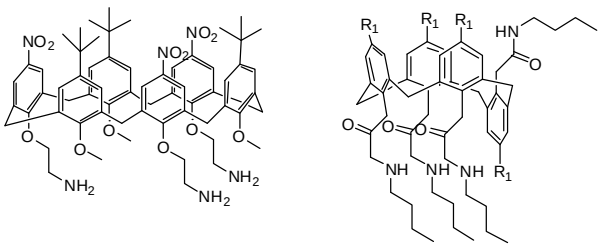


Fig.19 *Resorcin[n]arenes **17** and **18** with various substituents. Molecule **19** represents the building block for all calix[n]arenes.*

cucurbits (cucurbit[n]uril, **22,** Fig. 21) represent a relatively new, but rapidly growing class of artificial channel-forming molecules. Resorcin[n]arenes (**17**, **18**) form ohmic cation channels in lipid bilayer. The aggregate channel structure rather than tubular structure was proposed as an active form because these molecules form by themselves the restrictive portal for guest molecules.[2]

Calixarenes (Fig.19, **19**) are based on cyclic structures of diaryl methanes in 1,3-alt conformation decorated with various substituents (alkyl chains, sterols, alkylamides). The number of repeat units can vary from 4 to 6 (Fig.19). Depending on the substituent R_1 the properties can vary as well. The molecule **20** (Fig. 20, left) forms aggregate ion channels in lipid membranes and exhibits no ion selectivity. On the contrary, the molecule **21b** (1,3-*alt*-calix[4]arene tetraamide,[75] *paco*-form, Fig.19, right) can transport Cl^- very efficiently through lipid membranes. If the R_1 substituent is *t*-Bu (**21a**, *paco*-form) the channel is inactive.[76]



20 **21a R_1= *t*-Bu** **21b R_1= H**

Fig. 20 ***Left****: Three examples of calix[n]arene molecules. The structure of the molecule* ***20*** *exhibits channel activity with an aggregate channel structure.* ***Right****: Two examples of 1,3-alt-calix[4]arene tetraamide (paco-form). Molecule* ***21b*** *exhibits anion selectivity for Cl^- ions.*

Similar results but lower activities were observed for a *cone* analogue of compound **21b** (Fig. 20).[77] Simply by modulating the R_1 substituent in **21,** different activities of the artificial channel can be observed. Two molecules **20** which creates a 2.6 nm long nanotube and 1.6 nm wide, can act as a host and forms a supramolecular pseudorotaxane complex with 4.4'-bipyridinium (viologen) through efficient self-assembly in solution.[78]

Cucurbit[n]urils **22** (CB[n], Fig. 21) are a class of macrocycles based on methyl-bridged glycoluril (acetyleneurea) polymers, where "*n*" can vary between 5 and

10, and even dimers n=10/n=5 can be observed.[79] The cucurbit family acts mostly as host-guest complexes comparable with cyclodextrines[80] and crown-ethers.[80] They also show chiral recognition, a property readily achieved with chiral cyclodextrines.[81]

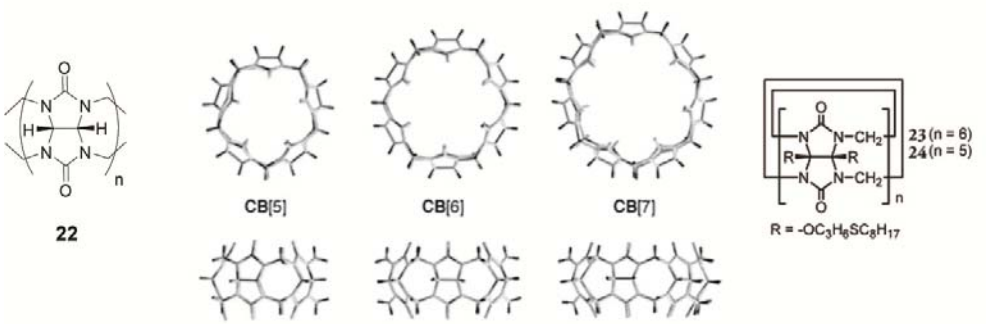


Fig. 21 ***Left****: General structure of cucurbit[n]urils (**22**). **Middle**: Three examples of cucurbit [5, 6, 7] uril (top and side view). **Right**: Functionalized cucurbits with long alkyl chains (**23**, **24**). Adopted from ref 79.*

Kim and co-workers have found a method to functionalize cucurbits[82] and created artificial channel systems with excellent ion selectivities. Cucurbit **23** (Fig. 21, right) showed proton selectivity in liposome experiments using the internal pH sensitive fluorescent 8-hydroxypyrene-1, 3, 6-trisulfonate (HPTS) dye. However, when acetylcholine was added which is known to form a stable host-guest complex with cucurbit[6], the fluorescence quenching was completely blocked. This result supports the theory that **23** is involved in proton transport across the membrane.[83]

1.4.3. Aggregate ion channels. How simple can a molecule be and still form a channel.

Aggregate ion channels are based on the principles and behavior of the natural channel forming amphotericin. The active channel combines antibiotic amphotericin and sterols arranged in two half-channel sections in the two bilayer leaflets.[2]

This type of complex hierarchical self-assembly is challenging. The promising approach involves "*bolaamphiphiles*", or two-headed amphiphiles, which form head to head structures. Molecules **8** and **9** (Fig. 13) are simple examples of this class of compounds. The central crown-ether in these structures is in this case not the ion recognizing unit of the molecule; it serves only to hold the structure together and has a limited functional role.[2] Simplified molecules **25** and **26** (Fig. 22) were prepared and showed more reliable channel formation than molecule **8**.[84, 85]

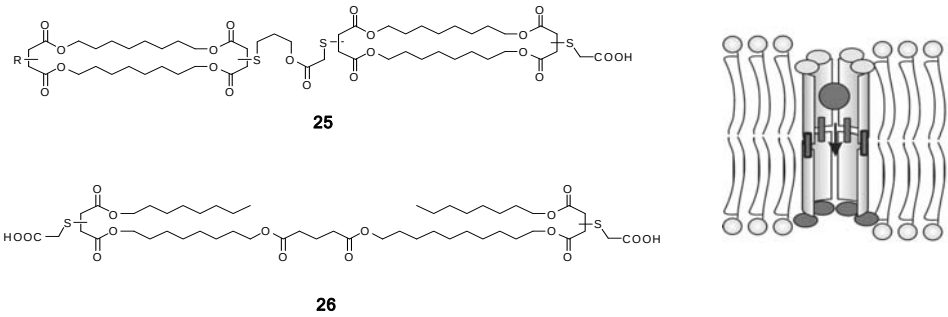


Fig. 22 *Compounds **25** and **26** (left) which aggregate to form ion channels (right). Adopted from ref 2.*

An important question is if, an even simpler molecule can form an active and functional artificial channel? Molecule **27** (Fig. 23) forms rosette-like structures (for detailed information about rosette structures in lipid bilayers, see chapter 5 of this thesis) in lipid bilayers with a hydrophilic interior and a hydrophobic exterior. The tube formed by these rosettes is stabilized by hydrogen bonds in the plane of the rosette.[86, 87]

Isophthalic acid analogue **28** (Fig. 23) also forms channel-like structures in PC/PS/cholesterol (phosphatidylcholin/phosphatidylserin/cholesterol) membranes.

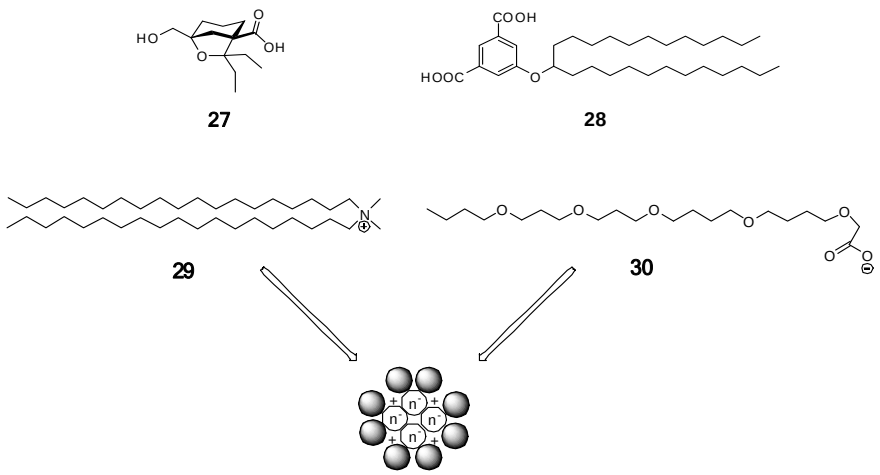


Fig. 23 *Simple organic molecules which form artificial ion channels in lipid bilayers. Molecules **29** and **30** form a neutral ion pair in the membrane and this leads to the channel formation (bottom). Adopted from ref 89.*

Surprisingly an even simpler system is able to form channels. The comprise ion pair of cationic and anionic amphiphiles **29** and **30** (Fig. 23) is such a system.[88, 89] The tetra-alkylammonium compound **29** and the carboxylate form of **30** form a neutral ion pair in the membrane and this leads to an ion-channel active assembly. The channel openings have uniform characteristics and, in all functional respects, appear to be like the channels formed by more complex structures.[39]

Further examples of artificial channels are those based on commercial surfactants,[90] metal-organic scaffolds,[91] polymers which induce pore formation in lipid bilayers,[92] porphyrines[93] and even systems based on metal organic polyhedras.[94]

1.5. Conclusions

Membrane transport is prerequisite for all living organisms. Channels and pores were formed during the evolution to fulfill this requirement. Reaching the complexity of the biological channels and pores is still difficult. On the other hand, functionality achieved by synthetic channels and pores can be compared to the natural ones. A number of successful artificial systems were reviewed in this chapter, but many opportunities do still exist. The development of new antimicrobial agents against increasing resistance of many bacteria to classical antibiotics stimulates the development of new membrane-active compounds as well as the development of new and innovative drug delivery systems. Treatment of human diseases like cystic fibrosis with synthetic chlorine channels[95] is another example of the application of synthetic channels and this holds also for the implementation of the artificial channels and pores into sensing devices.[96]

1.6. Aim and outline of this thesis

The aim of this thesis is to prepare artificial channels and pores by modifying natural channels with organic molecules or by synthesizing new channels and pores from organic starting materials.

In Chapter 2, the modification of a naturally-occurring channel-forming alamethicin molecule with azobenzene switches will be discussed as well as the properties of newly prepared artificial channels.

In Chapter 3, the functional size of the SecYEG translocation pore will be studied by attaching rigid spherical tetra-arylmethanes into *Outer membrane protein A*.

In Chapter 4, *Mechanosensitive Channel of Large Conductance* (MscL), a membrane protein channel that responds to increasing tension in the membrane by opening to form a pore with a 3 nm in diameter has been modified with a redox

thiaxanthylidene switch to become responsive to changes in redox potential. The same channel will be modified with multicharged spermine molecule to become responsive to a pH change of its environment.

In Chapter 5, the synthesis of an artificial channel from carbazole and phenanthrene building blocks will be described as well as the properties of the new molecule and the channel formation in lipid bilayers.

And finally in Chapter 6, the synthesis of pyridyl-containing diarylethene switch will be described as well as the magnetic properties of the newly prepared iron (II) complex with this switch.

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